Post-Inhibitory Rebound by δ-Cells Transforms Inhibition into Excitation and Redefines Islet Plasticity.

Authors

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Keywords

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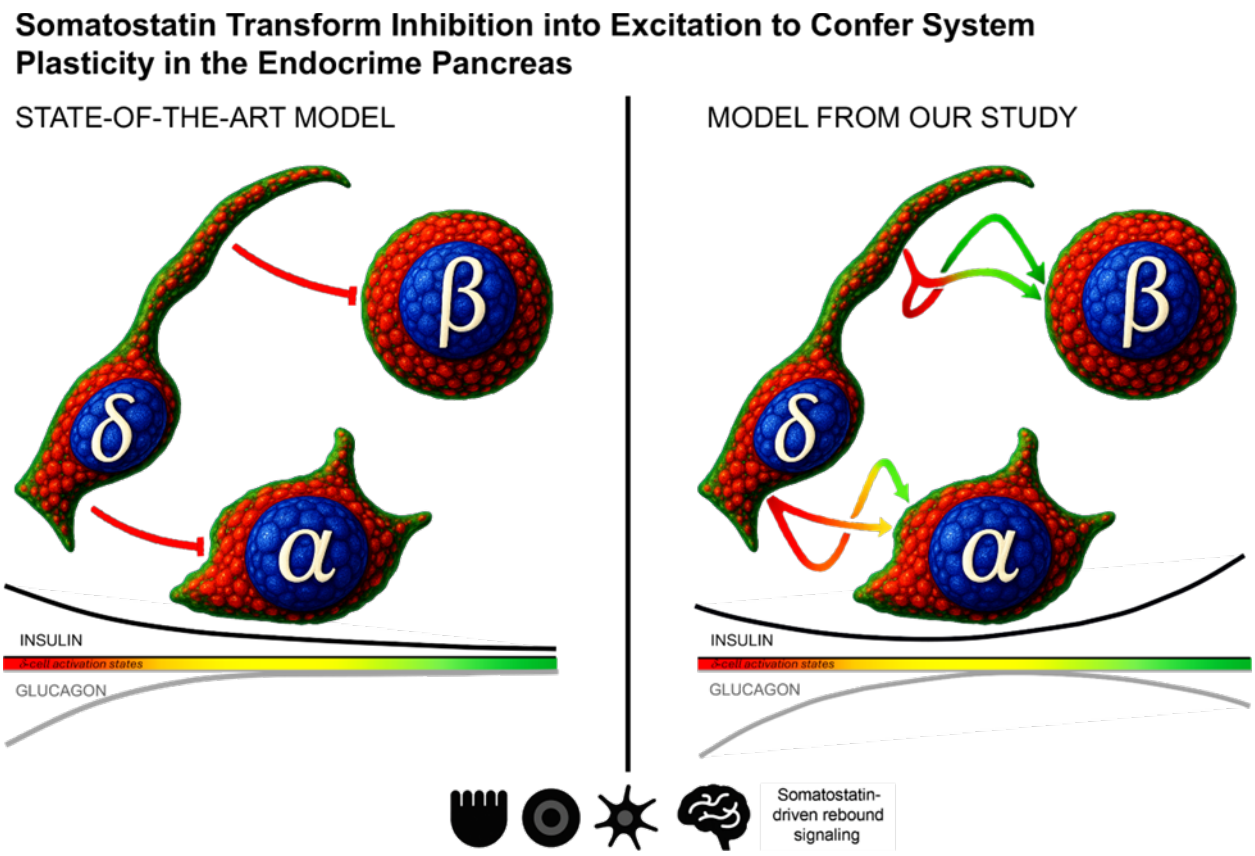
Abstract

The coordination of hormone secretion within the pancreatic islet remains incompletely understood. Here we identify post-inhibitory rebound (PIR), a transient excitatory overshoot of glucoregulatory hormones following inhibitory signaling within the islet, as a fundamental and previously unrecognized feature of islet physiology, further dissected through δ-cell–specific chemogenetic modulation. Human islets from donors with type 2 diabetes displayed concomitantly elevated insulin and somatostatin secretion with tightly correlated dynamics, a pattern reproduced in islets from high-fat-diet–fed mice. Chemogenetic δ-cell activation and silencing revealed that inhibitory δ-cell signals consistently trigger rebound excitation of β- and α-cells, generating glucose-dependent, synchronized oscillations of insulin and glucagon release that are partially mediated by somatostatin receptors. Physiological δ-cell stimuli, including urocortin-3 and ghrelin, elicited comparable rebound responses, linking this mechanism to native signaling pathways. In vivo, selective δ-cell modulation in islet grafts bidirectionally regulated systemic glucose tolerance, confirming δ-cell control over endocrine output. Beyond the pancreas, this work broadens somatostatin biology, revealing rebound excitation as a conserved motif across neuroendocrine, proliferative, inflammatory, and sensory systems. By reframing somatostatin from static inhibition to rhythmic modulation, these findings show that somatostatin confers system plasticity when needed, establishing δ-cell–driven PIR as a unifying principle of adaptive excitability and endocrine resilience.

Significance Statement

Hormone secretion in the pancreatic islet has long been explained by inhibitory feedback loops, with somatostatin viewed as a static suppressor of insulin and glucagon release. Our study overturns this paradigm by identifying post-inhibitory rebound (PIR) as a dynamic property of intra-islet signaling. δ-cell–driven PIR synchronizes α- and β-cell activity, enabling adaptive, glucose-dependent hormone release and revealing that somatostatin confers system plasticity when needed. This discovery reframes inhibition as a rhythmic and regenerative force, positioning PIR as a conserved mechanism of excitability relevant to neuroendocrine regulation, metabolic resilience, tumorigenesis, and sensory adaptation.



Graphical Abstract:

δ-cell activity dynamically shapes the insulin: glucagon ratio through somatostatin-mediated inhibition followed by post-inhibitory rebound (PIR) excitation. The red-to-green scale represents increasing δ-cell activation, from chemogenetic control *in vitro* to physiological modulation *in vivo,* linking hormonal balance across basal and altered or pathological somatostatin secretion in elevated glucose and prandial states. By transforming inhibition into rhythmic excitation, somatostatin confers adaptive plasticity for endocrine resilience. It exemplifies a conserved mechanism relevant to other somatostatin-rich systems, including gut, tumor, inflammatory, and neural contexts.

INTRODUCTION

The maintenance of blood glucose within a narrow physiological range is essential for survival (Röder et al., 2016; Matschinsky & Wilson, 2019). Glucose is the predominant fuel for most cells, and its availability fluctuates with feeding–fasting cycles, activity, and metabolic demand (Morton et al., 2014). Despite these fluctuations, systemic glucose balance is maintained through coordinated regulation by the liver, skeletal muscle, adipose tissue, and hypothalamus (Schwartz et al., 2013). Central to this network is the pancreatic islet of Langerhans, the body's principal glucose sensor (Gao et al., 2021; Noguchi & Huising, 2019).

Within the islet, α-, β-, and δ-cells form a densely interconnected network integrating hormonal, neural, and metabolic cues to orchestrate systemic glycemia (Hauge-Evans et al., 2009; Benninger & Piston, 2014; Rodriguez-Diaz & Caicedo, 2014). β-cells secrete insulin to promote glucose uptake and storage, while α-cells release glucagon to stimulate hepatic glucose production. Their secretory patterns are pulsatile, a hallmark of efficient endocrine signaling (Hellman, 2009; Edgerton et al., 2021). Oscillatory release into the portal vein maximizes hepatic responsiveness while maintaining peripheral glucose stability (Ferrell & Ha, 2014). Disruption of pulsatile insulin secretion is among the earliest abnormalities in type 2 diabetes, preceding β-cell loss or insulin resistance (Matthews et al., 1983; Matveyenko et al., 2012; Satin et al., 2015). Yet the mechanisms generating and synchronizing these rhythms in vivo remain poorly understood (Henquin, 2000; Hill & Hill, 2024).

Islet function critically depends on intra-islet communication, providing feedback to coordinate hormone output across cell types (Noguchi & Huising, 2019; Hauge-Evans et al., 2009). Each endocrine population acts as both signal generator and receiver: β-cells respond to glucagon, α-cells to insulin and somatostatin, and δ-cells to all three plus autonomic inputs (Pipeleers et al., 1985a; 1985b; Gao et al., 2021). These interactions create a self-regulating microcircuit that integrates multiple streams of information to maintain the glycemic set point. Even subtle alterations can shift insulin or glucagon thresholds, predisposing to dysglycemia and accelerating diabetes progression (Weir & Bonner-Weir, 2004; Fonseca, 2009).

Among endocrine cell types, δ-cells have historically received the least attention. Representing 5–10% of islet cells, they secrete somatostatin (SST), a peptide inhibiting both insulin and glucagon via Gi/o-coupled receptors throughout the islet (Strowski et al., 2000; Strowski & Blake, 2008; Hauge-Evans et al., 2009). Traditionally viewed as suppressive elements restraining excessive hormone release, δ-cells are increasingly recognized as versatile integrators. Their dense innervation, particularly by cholinergic fibers, and positioning at α/β interfaces suggest a role in sensing local activity and modulating network output in real time (Rodriguez-Diaz et al., 2011; Arrojo e Drigo et al., 2019).

Beyond inhibition, δ-cells act as temporal coordinators of islet function, influencing both magnitude and timing of secretion to synchronize insulin and glucagon oscillations (Rutter, 2009; Satin et al., 2015). By modulating hormone timing, δ-cells contribute to the pulsatility essential for glucose regulation (Benninger et al., 2014; Bertram et al., 2023). They integrate

metabolic, paracrine, and neural signals to ensure coherent endocrine responses across the islet (Rodriguez-Diaz et al., 2018; Gao et al., 2021; Noguchi & Huising, 2019).

A growing body of evidence has revealed paradoxical aspects of δ-cell function. While SST potently inhibits insulin and glucagon secretion in isolated settings, manipulations of δ-cell activity in vivo have produced inconsistent outcomes (Kailey et al., 2012; Flock et al., 2007). Activation, inhibition, or even ablation of δ-cells can yield net increases in hormone secretion, improved metabolic readouts, or both—observations that cannot be reconciled with a purely suppressive model (Hauge-Evans et al., 2009; Arrojo e Drigo et al., 2019; Briant et al., 2018; Huang et al., 2024; Li et al., 2018). For example, δ-cell activation can rapidly alter paracrine control of β-cells (Briant et al., 2018), whereas inhibition or ablation of δ-cells or SST disrupts islet coordination and impairs glucose homeostasis (Huang et al., 2024; Li et al., 2018; Miranda C., et al, 2025). These findings highlight the dynamic and context-dependent nature of δ-cell function, suggesting their role extends beyond inhibition.

In type 2 diabetes (T2D), δ-cell activity appears dysregulated: SST levels are altered, yet the consequences for islet output are complex and context-dependent (Singh et al., 2007). A recent study reported a significant reduction in δ-cell number in T2D islets, suggesting decreased SST production and impaired regulatory control of insulin and glucagon (Kothegala et al., 2023). Conversely, other work demonstrated increased glucose-induced SST secretion in T2D islets, indicating heightened δ-cell responsiveness under pathological conditions (Yang et al., 2025). Together, these observations underscore the complexity of δ-cell regulation in diabetes and highlight the need to redefine their contribution to islet physiology.

This gap is particularly pressing given the central role of intra-islet communication in maintaining metabolic balance and the glycemic set point (Rodriguez-Diaz et al., 2018; Huang et al., 2024). If δ-cells function as integrators of paracrine, neural, and possibly immune signals, their role extends to orchestrating the temporal structure of hormone release (Gao et al., 2021; Noguchi & Huising, 2019; Hauge-Evans et al., 2009). In this framework, δ-cells could regulate not only the magnitude but also the rhythm of insulin and glucagon secretion in response to changing metabolic demands (Rutter, 2009; Satin et al., 2015). Such a role would resolve long-standing paradoxes and open therapeutic opportunities in T2D, where disrupted oscillations and thresholds contribute to disease progression (Meier & Bonadonna, 2013).

Here, we interrogated this gap using human islet studies, revealing unexpected co-secretion patterns of insulin and SST that highlight complex δ-cell dynamics under physiological and pathological conditions. To understand the mechanistic basis of these observations, we applied chemogenetic tools for precise manipulation of δ-cell activity in vitro and in vivo, coupled with high-resolution islet output measurements. These experiments uncovered rebound dynamics, whereby transient SST inhibition paradoxically potentiates subsequent α- and β-cell responses. Our findings extend previous observations of δ-cell dysregulation in T2D (Singh et al., 2007; Salehi et al., 2007; Yang et al., 2025) and identify post-inhibitory rebound (PIR) as a novel δ-cell–driven response. PIR reframes δ-cells not as passive suppressors but as orchestrators that recalibrate glucoregulatory hormone levels and ratios—determinants central to glucose metabolism.

By identifying δ-cell–driven rebound dynamics as a regulator of hormone coordination, this study expands the concept of somatostatin signaling beyond inhibition to encompass temporal control of secretion and response. Because somatostatin acts as a conserved modulator across neuroendocrine, gastrointestinal, immune, and even neoplastic contexts, this role may extend well beyond the islet. These findings position δ-cells as prototypes of broader somatostatin-driven timing mechanisms that could influence rhythmic or adaptive processes in diverse physiological and pathological settings.

RESULTS

Impaired Glucose Responsiveness with Elevated Basal and Stimulated Secretion in Islets from T2D Individuals and HFD Mice

The pancreatic δ-cell has classically been viewed as an inhibitory node within the islet of Langerhans, exerting paracrine suppression of both α- and β-cells through secretion of somatostatin, a potent inhibitory peptide acting primarily via SSTR2 and SSTR5 receptors (Strowski & Blake, 2008; Vergari et al., 2019; 2020). By constraining insulin and glucagon release, δ-cells help fine-tune islet hormone ratios and preserve metabolic balance. Given that disordered secretion of both insulin and glucagon contributes to type 2 diabetes (T2D) pathophysiology, we investigated whether δ-cell activity and somatostatin secretion are altered under diabetic conditions.

To address this, we performed high-resolution perifusion experiments in human islets isolated from non-diabetic (ND) lean donors and from individuals with T2D (Figures 1, S1). Insulin and somatostatin were measured simultaneously across a glucose ramp (3 → 11 mM) to capture both basal and glucose-stimulated secretion. In ND islets, hormone release displayed the expected dynamic profile: low basal secretion with robust glucose-stimulated insulin secretion (GSIS) and a more modest somatostatin response. By contrast, T2D islets displayed a striking phenotype—both basal and stimulated secretion of insulin and somatostatin were markedly elevated compared with ND islets. Importantly, while ND islets showed no correlation between basal insulin and somatostatin release, secretion in T2D was tightly coupled, indicating coordinated hyperactivity of β- and δ-cells. Despite this hypersecretion, total islet content of insulin and somatostatin was reduced in T2D, whereas glucagon content remained unchanged. These findings indicate a state of β- and δ-cell hyperexcitability relative to α-cells, in which elevated output is paradoxically accompanied by depleted hormone stores.

This hypersecretory trend was recapitulated in murine models. Islets and pancreatic slices from high-fat diet (HFD)-fed mice showed elevated basal secretion of both insulin and somatostatin compared with regular chow-fed (RD) controls (Figure S2). These results demonstrate that δ-cells are responsive not only under physiological glucose stimulation but also to pathological perturbations such as diet-induced metabolic stress, thereby actively shaping islet hormone output.

While these observations highlight an unexpected coupling between insulin and somatostatin secretion in T2D and obesity, they do not fully resolve the mechanisms driving δ-cell hyperactivity. Current models of intra-islet communication propose that δ-cell activation may reflect either (i) a compensatory feedback response to heightened β-cell activity, or (ii) a failure of inhibitory regulation in pathological states, leading to unsuppressed β-cell and δ-cell secretion (Rorsman & Huising, 2018; Noguchi & Huising, 2019; Hartig & Cox, 2020; Hill & Hill, 2024). Alterations in somatostatin signaling pathways have been reported in these settings (Cejvanovic et al., 2021; Oduori et al., 2020; Yang et al., 2025), underscoring the incompletely understood but central role of δ-cells in governing islet homeostasis.

Dynamic Interplay of β–α–δ Cells Revealed by Chemogenetic Manipulation

To dissect how δ-cells modulate islet communication, we employed a chemogenetic strategy enabling cell-type–specific activation. Using Cre/loxP technology, we generated transgenic mouse lines expressing the excitatory Designer Receptor Exclusively Activated by Designer Drug (DREADD), hM3Dq, selectively in β-cells (INS-Cre), α-cells (GCG-Cre), or δ-cells (SST-Cre). Administration of clozapine-N-oxide (CNO) selectively activated canonical Gq signaling in targeted cells. Three F1 crosses were established: INS-DREADD Gq (β-cells), GLUCAGON-DREADD Gq (α-cells), and SOMATOSTATIN-DREADD Gq (δ-cells). Immunocytochemistry confirmed cell-specific expression and plasma membrane localization of HA-tagged hM3Dq (Figure 2 A, E, I).

In perifusion assays, CNO stimulation elicited robust, dose-dependent increases in homotypic hormone secretion—insulin, glucagon, or somatostatin—depending on the targeted cell type (Figure S3). Maximal responses were observed at 10 μM CNO, whereas partial desensitization occurred at 100 μM (Figure 2B, 2F, 2J), as quantified by AUC analysis (Figure 2C, 2G, 2K). Notably, hormone release was further potentiated under high-glucose conditions (16 mM). Even α-cells, typically suppressed at such glucose concentrations, displayed strong glucagon secretion upon chemogenetic activation (Figures 2 and S3).

Paracrine and Rebound Excitation: A Hidden Axis of Islet Regulation

CNO-driven activation of specific islet cell types produced both immediate "on-responses" in the targeted population and delayed heterologous "off-responses" in neighboring cells. For instance, stimulation of α-cells in GLUCAGON-DREADD-Gq islets not only boosted insulin secretion from β-cells but also enhanced somatostatin release from δ-cells, consistent with well-established paracrine interactions (Figure 2H). In contrast, β-cell chemogenetic activation did not trigger secondary somatostatin secretion from δ-cells during the late phase of insulin release under high-glucose conditions (16 mM; Figure 2D). This outcome was unexpected, since β-cell activation is widely known to stimulate δ-cells and drive somatostatin release, forming a classic inhibitory feedback loop.

Unexpectedly, we also detected "off-responses" in the form of post-inhibitory rebound (PIR) excitatory patterns that diverged from the canonical inhibitory paradigm. Activation of δ-cells in SOMATOSTATIN-DREADD-Gq islets initially suppressed both insulin and glucagon secretion, yet this was followed by strong rebound surges that resulted in a net increase in hormone output (Figure 2L). Likewise, β-cell activation paradoxically amplified glucagon release under high glucose, suggesting that transient δ-cell–mediated inhibition may prime both α- and β-cells for PIR (Figure 2D). Together, these findings identify PIR as a previously underappreciated mechanism of islet regulation`, particularly under conditions of elevated glucose or prolonged stimulation.

To confirm that these effects were directly attributable to δ-cell activation, we validated selective expression and functional engagement of DREADD-Gq across two additional independent F1 SOMATOSTATIN-DREADD-Gq mouse lines (Figure S4). In all lines, CNO stimulation consistently evoked acute somatostatin release, followed by rebound increases in insulin and

glucagon secretion with characteristic temporal delays relative to δ-cell output. This reproducibility across multiple genetic backgrounds underscores that the rebound dynamics are intrinsic to δ-cell–mediated inhibition rather than line-specific artifacts

Chemogenetic δ-Cell Inhibition: Revealing Asymmetry in Islet Control

To evaluate the impact of δ-cell suppression, we generated F1 SOMATOSTATIN-DREADD-Gi mice expressing the inhibitory hM4Di receptor. CNO administration reduced somatostatin secretion by ~60% (Figure 3A, D, E; Figure S5A, D, E). Remarkably, this led to a pronounced increase in glucagon secretion under high glucose (16 mM), a condition normally considered non-permissive for α-cell activity. A similar, though less surprising, increase was also observed under low glucose (1 mM) (Figure 3F–H, L; Figure S5F–H, L). In contrast, insulin levels were only modestly affected. These findings underscore that δ-cells exert a dominant regulatory influence on α-cell activity, while their modulatory effects on β-cells are more conditional and comparatively modest. Direct comparison of DREADD-Gq and DREADD-Gi manipulations further revealed that δ-cell activity not only tunes the overall insulin-to-glucagon balance but also orchestrates the temporal dynamics of islet output, with δ-cell activation promoting synchronized post-inhibitory rebound (PIR) in both α- and β-cells (Figure 3H–J; Figure S5H–J).

Delta Cell–Enabled Rebound Insulin Secretion is Glucose-Dependent and partially mediated by somatostatin

We next examined the glucose dependence of β-cell post-inhibitory rebound (PIR) following δ-cell activation. Perifusion of F1 SOMATOSTATIN-DREADD-Gq islets across a range of glucose concentrations (3, 7, and 16 mM) revealed that rebound insulin responses emerged only under permissive glucose conditions (7 and 16 mM), but not at basal (3 mM) glucose. The magnitude of these rebound responses was comparable to those achieved during first-phase glucose-stimulated insulin secretion or KCl-induced depolarization (Figure 3M, N). Pharmacological blockade of somatostatin receptors with cyclosomatostatin partially attenuated the PIR, demonstrating that somatostatin signaling contributes to—but does not fully account for—rebound excitation in β-cells (Figure 3O, P; Figure S6).

Native Ligands Reproduce δ-Cell DREADD Phenotypes

We evaluated whether natural ligands of delta cells, Urocortin-3 (Ucn3) and ghrelin, could reproduce the excitatory rebound phenomena observed with DREADD activation. Perifusion assays in C57BL/6J islets revealed that both ligands increased somatostatin secretion and induced biphasic insulin release, characterized by an initial inhibition followed by robust rebound (Figure 4). These findings provide a mechanistic explanation for the co-elevation of insulin and somatostatin observed in vivo and suggest that delta cells modulate islet hormone output dynamically, rather than functioning solely as inhibitory elements.

Physiological Impact of δ-Cell Modulation on Hormone Secretion and Glucose Homeostasis In Vivo

To assess the systemic consequences of δ-cell modulation, we transplanted islets expressing either activating (Gq) or inhibitory (Gi) DREADDs from male and female donors into the anterior chamber of the eye of STZ-diabetic female NUDE mice. Manipulations and evaluations were conducted in both recipient groups to probe potential sex-dependent effects of donor islets (Figures S7 and S8). As no major sex-specific differences were detected, the main figures present consolidated results. Engrafted mice restored normoglycemia within 30–40 days, showing comparable baseline glycemia across all groups and largely similar plasma levels of insulin, glucagon, and somatostatin under fasting, non-fasting, and feeding conditions (Figures 5 A-B3, 6A–E, S7).

In vivo imaging of GCaMP3-expressing δ-cells revealed robust, dose-dependent calcium responses upon sequential intraperitoneal CNO administration, confirming selective δ-cell activation within the grafts (Figure 5 C, D1-D3, E; Movie S1). Interestingly, these experiments consistently elicited a hypoglycemic effect. To explore this phenomenon further, parallel experiments measured glycemia and plasma hormone levels following sequential systemic administration of two escalating CNO doses (Figure 5 G-J).

Both previous and newly conducted experiments demonstrated that acute δ-cell activation consistently enhanced insulin secretion and lowered blood glucose during both short- and long-fasting conditions. In contrast, δ-cell inhibition had minimal effects on glycemia but increased glucagon secretion (Figures 6F–I, S8). During intraperitoneal glucose tolerance tests (IPGTTs), δ-cell activation promoted greater insulin release and improved glucose clearance relative to sham controls, whereas δ-cell inhibition produced subtler changes (Figures 6H, Figure S8 8.1A and S8.2A). Insulin tolerance tests (ITTs) revealed that δ-cell activation increased basal insulin secretion and conferred mild resistance to exogenous insulin-induced hypoglycemia without affecting glucagon levels, while δ-cell inhibition impaired glucagon counter-regulation without significantly altering glucose recovery (Figures 6I, S8 8.1B, 8.2B).

Together, these findings highlight the paradoxical yet physiologically significant role of δ-cell activation in enhancing islet hormone output and fine-tuning glucose homeostasis in vivo.

In Vivo δ-Cell Manipulation Confirms Perifusion-Derived Insights into Hormone Balance

To determine whether the regulatory effects of δ-cell activity observed in vitro (re-analyzed in Figure S9) extend to the physiological setting of feeding, we manipulated δ-cell function in vivo using DREADD-expressing islets transplanted into NUDE mice. CNO was administered chronically in drinking water to selectively activate or inhibit δ-cells during normal feeding. Consistent with perifusion experiments, both δ-cell activation and inhibition increased circulating levels of insulin and glucagon under postprandial conditions, but the balance of these responses diverged depending on the direction of δ-cell modulation (Figure 7A–C, A1–C1). δ-cell activation favored a coordinated increase in both hormones with a relative skew toward insulin, while δ-cell inhibition disproportionately elevated glucagon. These patterns mirrored the post-inhibitory rebound dynamics seen in vitro, underscoring the capacity of δ-cells to fine-tune the balance of islet hormone output during nutrient stimulation.

Analysis of plasma hormone ratios further emphasized this modulatory role. In CNO-treated mice with δ-cell activation, the insulin:glucagon ratio shifted upward, reflecting enhanced β-cell dominance, whereas δ-cell inhibition caused a marked reduction in this ratio due to excessive glucagon release (Figure 7A2–C2). Ratios involving somatostatin showed similar directional changes, confirming that δ-cell activity governs not only absolute hormone secretion but also their relative balance in circulation. Together, these findings validate the physiological relevance of δ-cell–mediated regulation, linking in vitro PIR dynamics to systemic hormone control in the context of feeding.

Summary

Our results fundamentally reshape the current understanding of paracrine interactions within the islet, adding a previously underappreciated layer of complexity. Delta cells, long considered primarily inhibitory elements, emerge as dynamic modulators capable of orchestrating not only the magnitude but also the temporal pattern and relative balance of islet hormone secretion. Activation of delta cells induces transient inhibitory effects on both alpha and beta cells, followed by pronounced post-inhibitory rebound (PIR) in insulin and glucagon release. In contrast, delta cell inhibition primarily lifts restraint on alpha cells, resulting in enhanced glucagon secretion with lesser impact on insulin.

These observations, consistent across in vitro and in vivo paradigms, suggest that delta cells function as central integrators within the islet, dynamically interpreting external and intra-islet cues to recalibrate hormone output. This perspective departs from static models of islet paracrine regulation, revealing that delta cell–mediated signaling can reshape the overall hormonal landscape in ways that may be unanticipated or fully predicted by traditional frameworks. Importantly, our findings support a novel conceptual model in which delta cells act as a hub linking cellular interactions to the net hormonal outcome—both the absolute levels of insulin, glucagon, and somatostatin and their relative ratios—allowing the islet to flexibly respond to integrated physiological signals.

This dynamic view of delta cell function not only refines our understanding of normal glucose homeostasis but also provides a novel mechanistic framework for interpreting dysregulated hormone patterns in pathophysiological states such as type 2 diabetes, highlighting the potential of delta cells as targets for therapeutic modulation. Figure 8 introduces this model, illustrating how delta cell–mediated interactions govern the temporal and quantitative orchestration of islet hormone secretion in response to diverse stimuli.

DISCUSSION

This study was motivated by a striking and unexpected observation: islets from individuals with type 2 diabetes (T2D) exhibit concurrent elevation of both insulin and somatostatin secretion at baseline and across higher glucose concentrations, with tightly correlated profiles—contrasting with the decoupled dynamics typically observed in healthy islets. In non-diabetic islets, basal hormone output is low, and glucose stimulation produces an asymmetric response: insulin rises steeply while somatostatin increases modestly, yielding a high insulin:somatostatin ratio that reflects β-cell dominance and restrained δ-cell tone (Kailey et al., 2012; Briant et al., 2018; Vergari et al., 2019; 2020). This asymmetry is essential for paracrine regulation of α- and β-cells and for systemic processes such as hepatic insulin clearance (Ipp et al,1987; Strowski & Blake, 2008). By contrast, the T2D phenotype—a lower insulin:somatostatin ratio with preserved or elevated δ-cell output (Rorsman & Huising, 2018; Vergari et al 2020; Oduori et al., 2020; Yang et al., 2025)—presents a paradox: how can δ-cell "dominance" coexist with hyperinsulinemia and inappropriate glucagon secretion in the setting of impaired β-cell competence?

Our work resolves this paradox by demonstrating that δ-cell activity can paradoxically prime and synchronize excitatory responses through post-inhibitory rebound (PIR), also referred to as post-inhibitory excitation (PIE). Chemogenetic and physiological manipulations showed that transient δ-cell activation suppresses insulin and glucagon but is consistently followed by rebound peaks. Such biphasic dynamics—acute inhibition followed by overshoot excitation—are hallmarks of PIR observed across excitable systems including thalamocortical circuits, brainstem respiratory networks, and cardiac pacemaker cells (Smith et al., 1991; Steriade et al., 1993; DiFrancesco, 1993; McCormick & Bal, 1997). PIR thus provides a mechanistic framework connecting our human islet data, mouse DREADD ex vivo perifusion dynamics and in vivo experiments, together with earlier studies showing rebound hormone release after somatostatin withdrawal (Leblanc et al., 1975; Ward et al 1975; Bratusch-Marrain et al, 1981).

Several convergent mechanisms underlie PIR in α- and β-cells, largely through Gi/o receptor signaling, the canonical pathway downstream of SSTRs. First, Gi/o activation suppresses cAMP production and downstream effectors such as PKA and EPAC, reducing granule priming, exocytotic competence, and ion channel facilitation (Kagimoto et al 1994; Doyle & Egan, 2007; Roed et al., 2012; Tengholm & Gylfe, 2017; Yu et al 2019; Dyachok et al., 2021; Dickerson et al., 2022). Relief of inhibition allows rapid cAMP recovery with transient overshoot, amplifying Ca^{2+}-triggered secretion. Second, Gi/o signaling induces hyperpolarization not only via GIRK channels but also through Src-family kinase–dependent Na^+/K^+-ATPase (NKA) activation, which suppresses excitability; rebound occurs when reduced NKA activity permits post-inhibitory depolarization and synchronized Ca^{2+} entry (Dickerson et al., 2022). Third, Ca^{2+} handling contributes: inhibition favors ER refilling, and upon release, store-operated Ca^{2+} entry (SOCE) and Ca^{2+}-induced Ca^{2+} release generate amplified cytosolic transients (Berts et al., 1997; Chen et al., 2003; Tian et al., 2011; Rorsman et al., 2012; Sabourin et al., 2015; Klec et al., 2019; Zhang et al., 2020). Finally, δ-cell somatostatin release is pulsatile and phase-locked to islet Ca^{2+} oscillations; release and withdrawal therefore synchronize rebound responses across α- and β-cells (Hauge-Evans et al., 2009; Zhang et al., 2020; Ren et al., 2022).

The rebound hypothesis also offers a mechanistic explanation for sustained insulin and somatostatin secretion in T2D. Chronic δ-cell activation—driven by hyperglycemia or altered paracrine feedback—may foster δ-cell hyperexcitability or enhanced tonic tone, sustaining insulin secretion through repetitive rebound cycles. PIR-like behavior in β-cells has been proposed to arise from "hyperexcitation" during prolonged glucose exposure, a state thought to underlie reversible insulin secretory defects (Nichols & Remedi, 2012). This may explain early hyperinsulinemia preceding β-cell failure (Weir & Bonner-Weir, 2004) and reconcile prior discrepancies: somatostatin may acutely suppress secretion while promoting pathological hypersecretion over longer timescales (Omar-Hmeadi et al., 2020; Yang et al., 2025).

Rebound dynamics extend to glucagon. Somatostatin inhibits α-cells as part of normal paracrine control (Gromada et al., 2007; Vergari et al., 2020). Yet relief of inhibition can generate amplified glucagon bursts, consistent with α-cell expression of Gi/o-coupled SSTR2 and α_2-adrenergic receptors (Salehi et al., 2007; Hellman et al., 2009; Zhang et al., 2013). Hyperpolarization via NKA reduces Ca^{2+} influx, and rebound depolarization reopens Ca^{2+} channels, triggering synchronized glucagon release. Pulsatile somatostatin may thus paradoxically potentiate glucagon secretion, explaining inappropriate dual hypersecretion in T2D.

Frequent δ-cell activation followed by inhibition amplifies rebound probability and magnitude, producing net rises in insulin and glucagon measured in vivo, including in high-fat-diet models. δ-cell "dominance" therefore reflects active shaping of secretion dynamics, not static suppression. DREADD-based manipulations support this model: selective δ-cell activation suppressed insulin and glucagon followed by rebound peaks resembling human studies (Leblanc et al., 1975; Ward et al 1975; Bratusch-Marrain et al, 1981). Conversely, δ-cell inhibition triggered glucagon disinhibition and smaller insulin rebounds, consistent with distinct α- and β-cell sensitivities and receptor expression (Kailey et al., 2012; Gromada et al., 2007). In vivo, δ-cell activation enhanced glucose tolerance in transplantation models; although systemic insulin and glucagon levels rose—particularly relative to somatostatin—the insulin:glucagon ratio remained balanced toward insulin dominance. By contrast, δ-cell inhibition in vivo markedly increased circulating glucagon and shifted the ratio toward glucagon dominance.

Our findings reveal that somatostatin-driven Gi/o signaling generates structured sequences of inhibition followed by post-inhibitory rebound, reconciling the paradoxical co-elevation of insulin and somatostatin in T2D. These insights extend beyond islets: PIE appears as a conserved regulatory motif across neuroendocrine and visceral systems, with implications for therapeutic targeting. Future directions include dissecting NKA and SOCE contributions to PIR, defining δ-cell timing changes under chronic metabolic stress, and testing temporally patterned δ-cell modulation as a strategy to restore physiological hormone rhythms and improve glycemic control.

Several additional considerations refine the PIR framework. Receptor subtype and coupling are central: in human α- and β-cells, SSTR5 and SSTR2 predominates and signals through Gi/o (Strowski and Blake, 2008; Vergari et al., 2019), dictating whether somatostatin action leads to GIRK-mediated hyperpolarization, NKA activation, or cAMP suppression. Variation in expression across species or disease states likely underlies differences in rebound amplitude and timing (Kailey et al., 2012; Roed et al., 2012). Other paracrine and hormonal cues also shape

PIR; in our study, both urocortin3 and ghrelin enhanced insulin rebound by modulating δ-cell activity and somatostatin release (van der Meulen et al., 2015; DiGruccio et al., 2016). Rebound dynamics are further tuned by sex and metabolic state, as seen in glucagon sensitivity. Beyond these, chronic disease remodeling—altered receptor expression, ion channel composition, gap junction connectivity (Briant et al., 2018), and ER/SOCE machinery—reshapes inhibitory tone and rebound capacity, contributing to the heterogeneity of T2D.

Our findings emphasize a dynamic consequence of δ-cell modulation beyond static inhibitory models, suggesting that timed δ-cell engagement may be harnessed to improve postprandial glucose control. Systemically, δ-cell activity influences hepatic insulin clearance and hormone kinetics (Blackard & Nelson, 1970; Efendic et al., 1975; 1976; Sherwin et al., 1976; Bonora et al., 1983; Ipp et al., 1987). Thus, improved glucose tolerance following δ-cell activation likely reflects both intra-islet PIR-mediated insulin augmentation and reduced hepatic clearance. Similarly, PIR-mediated glucagon enhancement may affect hepatic glucose production, exacerbating hyperglucagonemia and hyperglycemia in T2D. These systemic effects underscore the translational importance of δ-cell signaling, suggesting therapies targeting somatostatin pathways could modulate islet secretion, SSTR re-sensitization, and hormonal clearance dynamics.

Clinically, PIR reframes strategies for islet-targeted therapy. Somatostatin signaling contributes not only inhibitory control but also rebound-driven excitation of insulin and glucagon, reconciling paradoxical T2D observations and aligning with PIR mechanisms across excitable systems. δ-cells emerge as synchronizers of normal pulsatility and drivers of pathological hypersecretion. Historically, somatostatin analogs have blunted hormone hypersecretion in neuroendocrine pathologies, though effects are context-dependent (de Herder & Lamberts, 2003; Öberg et al., 2010; Wolin, 2012). In islets, strategies modulating timing rather than absolute δ-cell output may leverage rebound potentiation to enhance postprandial insulin without prolonged suppression or receptor desensitization. Conversely, chronic δ-cell hyperactivity inducing large rebounds could destabilize glucose homeostasis. DREADD and ligand-based tools offer a framework for exploring temporally precise interventions.

Limitations include incomplete understanding of NKA regulation by GPCR–SFK pathways in α- versus β-cells, a focus on acute dynamics with minimal longitudinal data, species differences in islet architecture and receptor expression, and the broader δ-cell secretome and intercellular interactions that may influence network timing and PIR. Despite these caveats, human perfused preparations and infusion studies suggest conserved rebound phenomena amenable to translational exploration (Leblanc et al., 1975; Ward et al 1975; Bratusch-Marrain et al, 1981).

In conclusion, δ-cells function as dynamic temporal orchestrators of islet excitability, shaping insulin and glucagon rhythms through rebound excitation. In healthy islets, transient δ-cell activation and somatostatin release synchronizes α- and β-cell activity for appropriate pulsatility (Salehi et al., 2007; Briant et al., 2017). In T2D, chronic δ-cell activation or SSTR desensitization may drive maladaptive rebounds, contributing to dysregulated glycemia. Analogous to inhibitory interneurons in neural networks, δ-cells suppress and coordinate excitatory output (Isaacson & Scanziani, 2011). Beyond islets, somatostatin-driven PIE represents a conserved regulatory principle across neuroendocrine, gastrointestinal, and sensory systems (Barbieri et al., 2013; Corleto et al., 2006; Hagströmer et al., 2006; Huang et al., 2018). Understanding this temporal logic opens avenues for δ-cell modulation in diabetes and broader PIE-based interventions in cancer, inflammation, and sensory physiology.

STAR Methods

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, **Dr. Rayner Rodriguez-Díaz (r.diaz4@edu.miami.edu)**.

Materials Availability

All unique mouse lines and reagents generated in this study are available from the Lead Contact upon reasonable request and execution of a material transfer agreement (MTA).

Data and Code Availability

All data supporting the findings of this study are provided in the paper and Supplementary Information. Raw perifusion time-course data and in-vivo metabolic datasets are available from the Lead Contact upon reasonable request. No custom code was used.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human Islet Donors

Human pancreatic islets were obtained from non-diabetic (ND) and type 2 diabetic (T2D) donors via the Integrated Islet Distribution Program and the Diabetes Research Institute Human Cell Processing Core (University of Miami) or purchased from Prodo Laboratories. Donor metadata (age, sex, BMI, HbA1c) are listed in Table S1. Islets were cultured overnight in CMRL-1066 (5.5 mM glucose) supplemented with 10% FBS, 2 mM L-glutamine, and 100 U/mL penicillin/streptomycin before experiments. Preparations with >80% viability and <20% exocrine contamination were used.

Human research compliance. De-identified human tissue was used in accordance with institutional guidelines and IRB policies for non-human subjects research/waived consent.

Mouse Models

For diet-induced obesity studies, C57BL/6J mice were maintained on standard chow (RD) or 45% high-fat diet (HFD; Research Diets D12451) for 30 days prior to islet isolation.

For chemogenetics, Cre drivers were crossed to Cre-dependent DREADD reporter lines to target specific endocrine subsets:

- **β-cell activation:** Ins2-Cre (JAX 003573) × ROSA-hM3Dq-HA-citrine (JAX 026220) → **INS-DREADD-Gq**
- **α-cell activation:** Gcg-iCre (JAX 030663) × ROSA-hM3Dq-HA-citrine (JAX 026220) → **GLUCAGON-DREADD-Gq**
- **δ-cell activation:** Sst-Cre (JAX 013044) × ROSA-hM3Dq-HA-citrine (JAX 026220) → **SOMATOSTATIN-DREADD-Gq**
- **δ-cell inhibition:** Sst-Cre × ROSA-hM4Di-HA-citrine (JAX 026219) → **SOMATOSTATIN-DREADD-Gi**

Additional δ-cell Gq lines used for imaging/reporters:

- **SOMATOSTATIN-DREADD-Gq; GCaMP3:** (Sst-Cre × ROSA-hM3Dq) × ROSA-GCaMP3 (JAX 029043)
- **SOMATOSTATIN-DREADD-Gq-mCherry:** Sst-Cre × ROSA26-hM3Dq-mCherry (JAX 026943)

Both sexes (2–3 months) were used unless stated otherwise. Genotypes were confirmed by PCR.

Animal ethics. Procedures were approved by the University of Miami IACUC and complied with NIH guidelines.

Sex as a biological variable. Sex was recorded for all donors and mice; analyses stratified by sex are reported where relevant.

METHOD DETAILS

Mouse Islet Isolation and Culture

Islets were isolated by collagenase P digestion, purified by density gradient, and hand-picked. For diet studies, islets were cultured 4 h before perifusion. For chemogenetic studies, islets were cultured overnight in RPMI-1640 (11 mM glucose, 10% FBS, antibiotics) prior to perifusion or transplantation.

Perifusion Assays and Chemogenetic Manipulations (in vitro)

Dynamic secretion was measured in parallel chambers (Biorep) at 37 °C with constant flow **0.06 mL/min** of KRB (in mM: 115 NaCl, 5 KCl, 24 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES; 0.1% BSA; pH 7.4).
Islets (**150 IEQ**/chamber) equilibrated 30 min, then underwent glucose ramps:

- **Mouse islets:** 3–16 mM or 3→16→1 mM
- **Human islets:** 3→11 mM

Chemogenetic stimulation. For cell-specific activation, DREADD-Gq islets received sequential **4-min** CNO pulses (1, 10, 100 µM) spaced **8 min** apart at either 16 mM or 1 mM glucose. For δ-cell inhibition, SOMATOSTATIN-DREADD-Gi islets received identical CNO pulse trains. Fractions were collected every **2 min**.

Insulin and glucagon were quantified by ELISA (Mercodia); somatostatin by ELISA (Phoenix Pharmaceuticals). Where indicated, **Cyclosomatostatin (500 nM)** was included at 16 mM glucose to test SSTR dependence of **post-inhibitory rebound (PIR)** insulin responses. KCl depolarization (25 mM) served as a normalization/positive-control stimulus. PIR insulin was assessed at **3, 7, and 16 mM** glucose.

Expected pharmacology (summary). DREADD-Gq produced dose-dependent homeotypic secretion (insulin, glucagon, or somatostatin) at permissive glucose, with occasional desensitization at 100 µM CNO; DREADD-Gi reduced somatostatin by ~60% and disinhibited glucagon.

Ligand Stimulation Studies (Endogenous δ-cell Activation)

Perifusion assays at **16 mM** glucose tested **Urocortin-3 (Ucn3)** and **Ghrelin (Ghr)** at **2.5 nM** and **25 nM** (pulsed). Both ligands increased somatostatin and reproduced the biphasic "inhibition-then-rebound" insulin secretion profile.

Immunocytochemistry and Confocal Imaging

Pancreata were fixed in 4% paraformaldehyde, cryoprotected, and cryosectioned (40 µm). Sections were stained with anti-HA (Cell Signaling 3724), anti-insulin (Abcam ab7842), anti-glucagon (Sigma G2654), anti-somatostatin (Abcam ab53165), Alexa Fluor secondaries, and DAPI. Confocal imaging (Leica SP8) confirmed cell-type specificity and membrane localization of HA-tagged DREADDs.

In Vivo Islet Transplantation and Engraftment

To isolate δ-cell contributions to systemic glucose control, **200 IEQ** from F1 donors were transplanted into the anterior chamber of the eye of **female athymic nude mice (10 weeks)** rendered diabetic with **streptozotocin (200 mg/kg, i.v.)**. Normoglycemia returned within 30–40 days. Recipients were grouped by donor genotype (SOMATOSTATIN-DREADD-Gq, SOMATOSTATIN-DREADD-Gi, or Sst-Cre controls) and by donor sex. Mice were maintained ≥90 days before testing.

In vivo δ-cell Ca^{2+} imaging. A subset received contralateral grafts from **SOMATOSTATIN-DREADD-Gq; GCaMP3** mice. Fluorescence responses to sequential i.p. CNO (**0.5 and 5 mg/kg**) were recorded with a custom laser-scanning setup and analyzed in ImageJ.

Metabolic Testing in Transplanted Mice

Testing was performed under: **postprandial** (2 h after feeding onset; ZT14), **short fasting** (2 h), or **long fasting** (16 h overnight). Glycemia (OneTouch Ultra) and plasma hormones (ELISA) were measured.

CNO Administration

For **acute** δ-cell manipulation, **CNO 5 mg/kg i.p.** was given **30 min** before tests; controls received saline. For **short-chronic** manipulation, CNO was provided in drinking water for 7 days (estimated **5 mg/kg/day**, assuming ~4 mL/mouse/day).

Intraperitoneal Glucose Tolerance Test (IPGTT)

Following 16 h fasting, mice received **2 g/kg** glucose i.p. Blood glucose was measured at -30, 0, 15, 30, 45, 60, 90, and 120 min. Hormone levels were estimated at -30, 0, 15, and 120 min. Each animal underwent two sessions with crossover (CNO vs saline).

Insulin Tolerance Test (ITT)

After 2 h fasting, mice received **0.75 IU/kg** insulin i.p. following saline or CNO pretreatment. Blood glucose measures mirrored the IPGTT, while glucagon was estimated at -30, 0 and 30 min.

Observed pharmacodynamics (summary). Acute δ-cell activation produced a transient hypoglycemia accompanied by elevated plasma insulin. During ITT, both activation and inhibition of δ-cells resulted in reduced plasma glucagon levels compared with controls. Notably, despite this suppression, the overall glucose counterregulatory response to insulin-induced hypoglycemia remained unaffected.

QUANTIFICATION AND STATISTICAL ANALYSIS

Perifusion traces were baseline-corrected and, when indicated, normalized to total hormone content and/or the KCl response. **Peak amplitude** and **area under the curve (AUC)** were computed for each stimulus. **PIR insulin** was quantified as AUC above baseline after CNO withdrawal (or relative to no-DREADD controls under identical stimulation).

Statistical analyses were performed in **GraphPad Prism 10**. Two-group comparisons used paired or unpaired two-tailed **t-tests** as appropriate. Multi-factor experiments used **two-way ANOVA** with **Šidák** post-hoc correction. Data are mean ± SEM; $\mathbf{p < 0.05}$ was considered significant.

Randomization and blinding. For in-vivo tests, animals were randomly assigned to treatment order (CNO vs saline crossover). Sample processing and ELISA quantification were performed with investigators blinded to group when feasible.

Exclusion criteria. Pre-specified criteria included perifusion duplicate failure or abnormally high or low hormone concentrations related to the data. Also, surgical failure to engraft (no reversion of hyperglycemia by day 40) or death during the experimental protocol. excluded cases are reported in figure legends where applicable.

KEY RESOURCES TABLE

(To be completed at final submission; will list antibodies, mouse strains/lines, ligands, CNO source, ELISAs with catalog numbers, software.)

Summary of Experimental Logic

Experimental Arm	Aim	Manipulation	Readouts
Human islet perifusion	Assess parallel insulin–somatostatin dynamics	Glucose steps	Hormone output & secretion kinetics
Mouse RD vs HFD islet perifusion	Test basal hypersecretion	Diet manipulation	Hormone output
DREADD-Gq / DREADD-Gi perifusion	Dissect δ-cell effects on α/β cells	CNO pulse trains	PIR and rebound dynamics
Intraocular graft model	Evaluate systemic relevance	CNO (acute/chronic)	Glycemia, plasma hormones, IPGTT/ITT
Ligand (Ucn3, Ghr) assays	Test endogenous δ-cell-driven PIR	Pulsed ligands	Rebound insulin secretion

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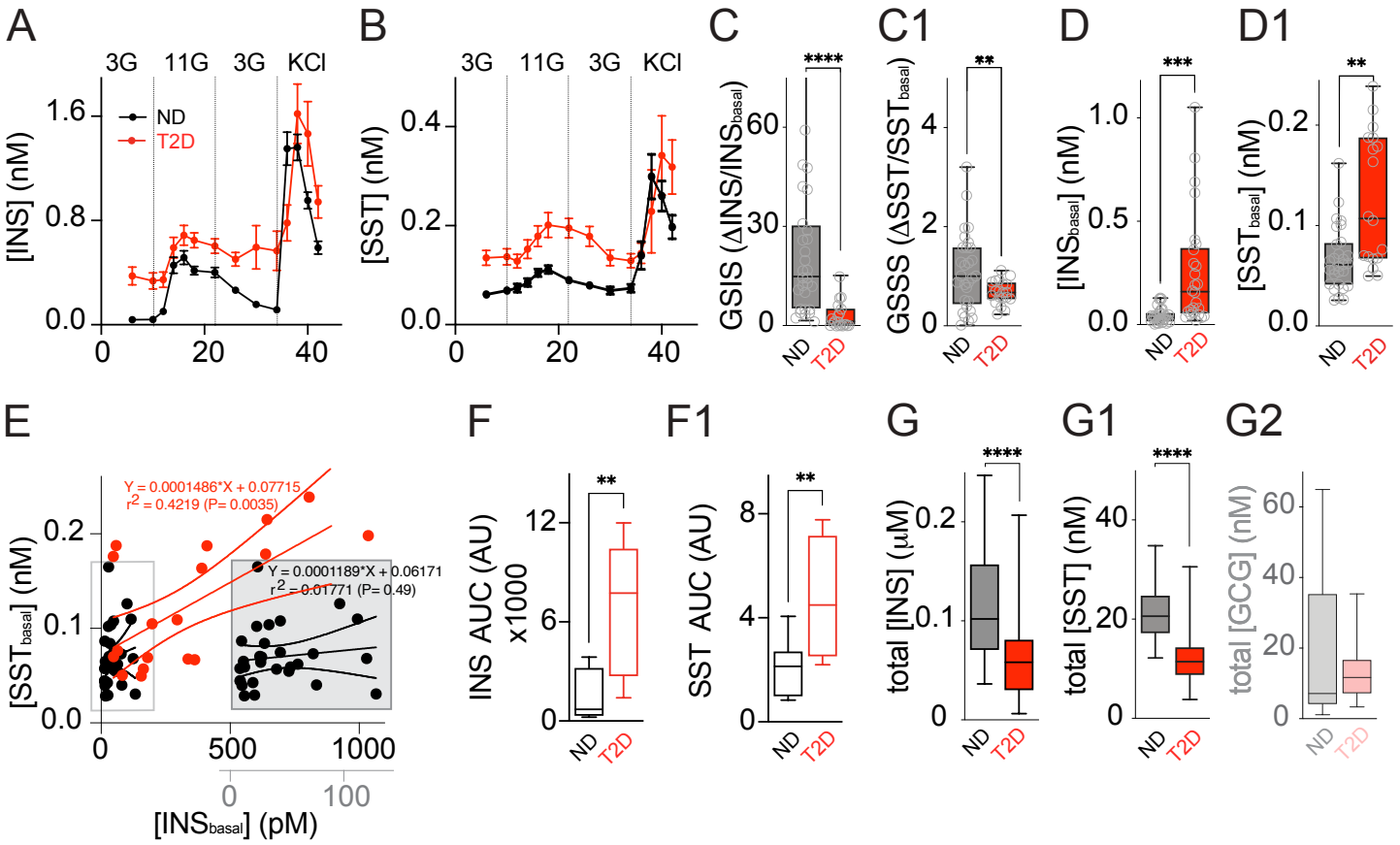


Figure 1. Type 2 Diabetic Human Islets Exhibit Elevated Basal Insulin and Somatostatin Secretion with Impaired Glucose Responsiveness

Isolated islets from type 2 diabetic (T2D) donors display abnormally high basal insulin and somatostatin secretion under low glucose (3 mM) compared with non-diabetic (ND) controls, accompanied by blunted glucose-stimulated hormone release despite increased absolute output.

(A, B) Dynamic perifusion profiles of insulin (A) and somatostatin (B) secretion in ND islets (n = 15; HbA1c < 5.6; BMI < 27) and T2D islets (n = 15; HbA1c > 6.0) during sequential exposure to 3 mM and 11 mM glucose. T2D islets show elevated basal secretion and diminished responsiveness to glucose compared with ND islets.
(C, C1) Quantification of glucose responsiveness, expressed as relative change from basal ($\Delta HORMONE_{stim}/HORMONE_{basal}$), for insulin (C, GSIS) and somatostatin (C1, GSSS). ND islets exhibit significantly greater fold responses than T2D islets.
(D, D1) Basal insulin (D, INS_{basal}) and somatostatin (D1, SST_{basal}) secretion at 3 mM glucose are tightly restrained in ND islets but significantly elevated in T2D islets.
(E) Linear regression of basal insulin and somatostatin secretion reveals no correlation in ND islets ($r^2 = 0.018$, P = 0.49), whereas T2D islets show a significant positive correlation ($r^2 = 0.422$, P = 0.0035), indicating dysregulated co-secretion.
(F, F1) Area under the curve (AUC) analysis of perifusion traces (A, B) quantifies total hormone release across glucose transitions (3 → 11 → 3 mM), showing significantly higher absolute secretion of insulin (F) and somatostatin (F1) in T2D islets compared with ND.
(G) Hormone content analysis reveals reduced total insulin (G) and somatostatin (G1) in T2D islets, despite their hypersecretion. Glucagon content (G2) was comparable between ND and T2D islets, serving as a reference control.

Statistical analysis: Two-way ANOVA for panels A and B; unpaired t-tests for panels C-D1,F-G2).

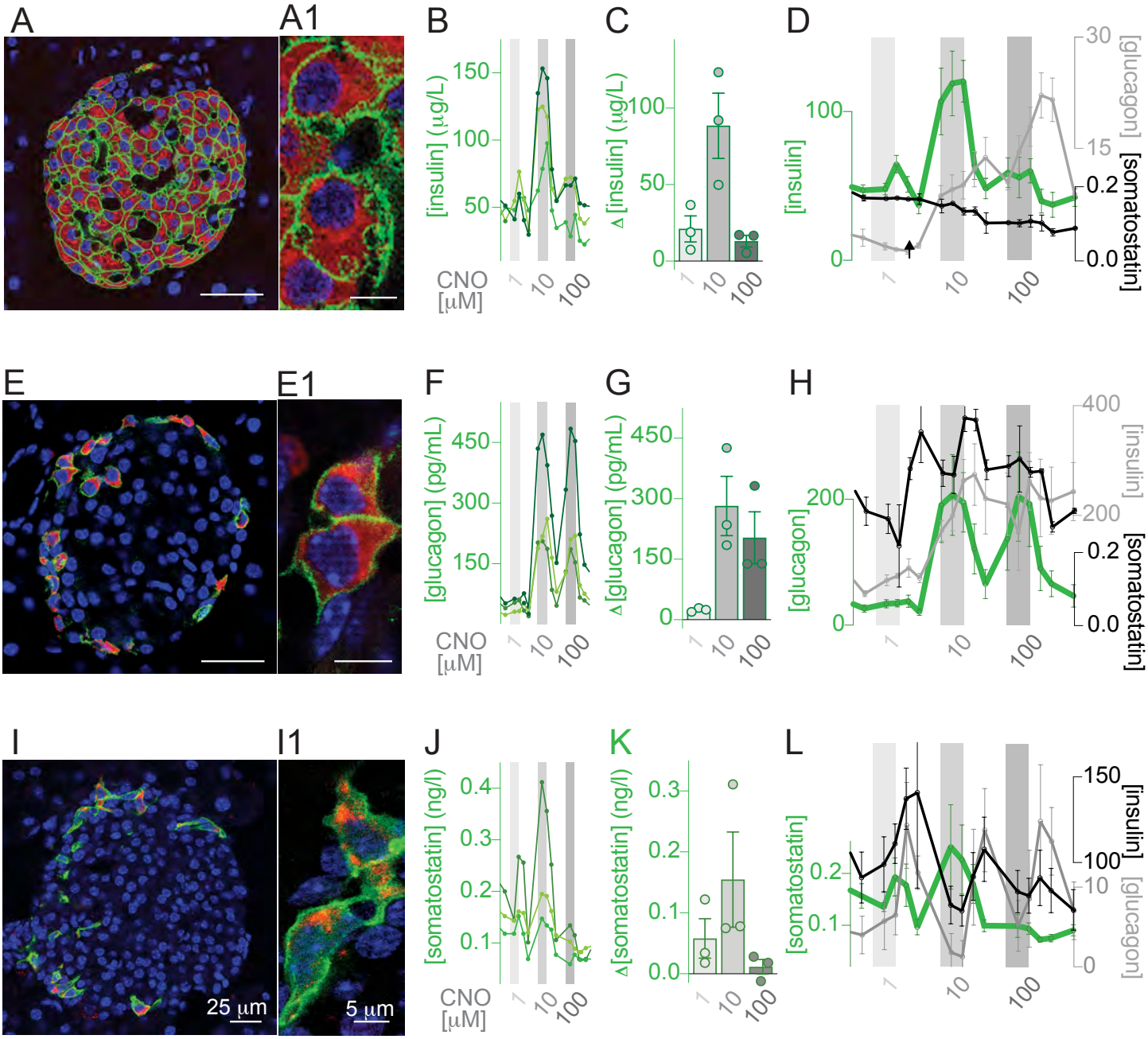
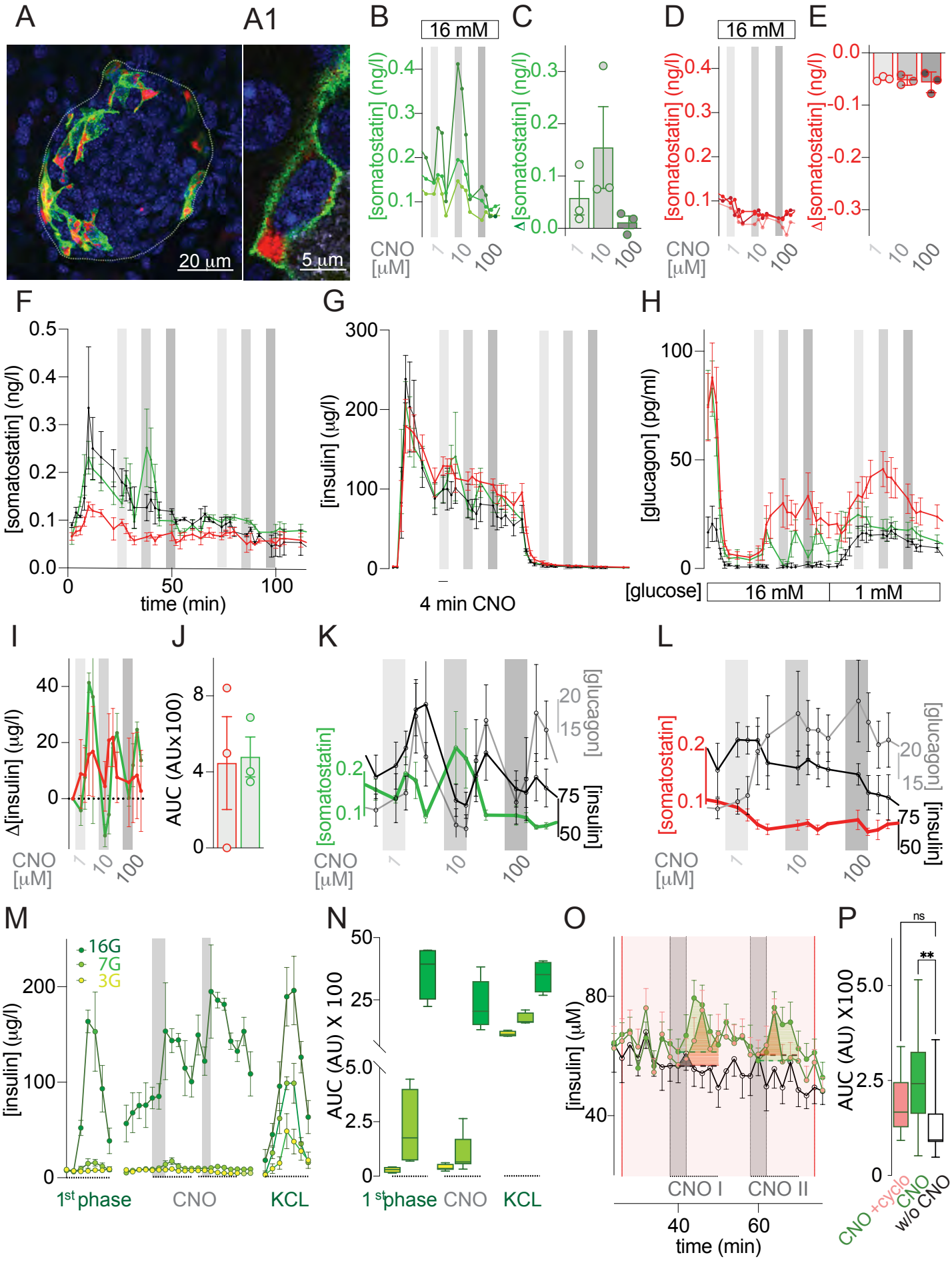


Figure 2. Cell-Specific Activation of DREADD-Gq Drives Dose-Dependent Idiotypic Hormone Secretion and Modulates Heterotypic Hormone Release in Islet Microcircuits.
Selective chemogenetic stimulation of DREADD-Gq in α-, β-, or δ-cells induces robust secretion of the corresponding idiotypic hormone—glucagon, insulin, or somatostatin, respectively—in a dose-dependent manner upon clozapine-N-oxide (CNO) administration. Subsequent changes in heterotypic hormone secretion are observed from non-targeted neighboring endocrine cell populations, occurring after CNO stimulation has ceased.

(A, E, I) Confocal images of pancreatic islets showing the distribution of α-, β-, and δ-cells within islets (scale bar, 25 µm).
(A1, E1, I1) High-resolution images highlighting HA-tagged DREADD-Gq reporter expression (green) restricted to insulin+ β-cells (A1), glucagon+ α-cells (E1), or somatostatin+ δ-cells (I1) (scale bar, 5 µm).
(B, F, J) Perifusion traces of idiotypic hormone secretion in response to sequential 4-minute CNO stimulations at 1, 10, and 100 µM under stimulatory glucose conditions (16 mM). Each trace represents an islet preparation

from an individual mouse (n = 3 per group).
(C, G, K) Quantification of idiotypic hormone secretion using area under the curve (AUC) analysis corresponding to the dose-response profiles in panels B, F, and J.
(D, H, L) Mean ± SEM hormone secretion profiles (n = 3 biological replicates per group) during 16 mM glucose perifusion, illustrating idiotypic hormone release (green) and concurrent dynamics of heterotypic hormone secretion (gray and black).

These data demonstrate that DREADD-Gq-mediated activation elicits specific hormone release from targeted endocrine cell types and consistently alters secretion from adjacent non-targeted cells, revealing functional crosstalk in islet cell networks.


A
A1
20 µm
5 µm
B
16 mM
[somatostatin] (ng/l)
CNO [µM]
C
Δ[somatostatin] (ng/l)
D
16 mM
E
F
[somatostatin] (ng/l)
time (min)
G
[insulin] (µg/l)
4 min CNO
H
[glucagon] (pg/ml)
[glucose]
16 mM
1 mM
I
Δ[insulin] (µg/l)
J
AUC (AUx100)
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16G
7G
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[insulin] (µg/l)
1st phase
CNO
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O
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CNO I
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AUC (AU) X100
CNO +cyclo
CNO
w/o CNO

Figure 3. Chemogenetic Modulation of δ-Cells Reveals Bidirectional Paracrine Control of Insulin and Glucagon Secretion via Somatostatin-Dependent Mechanisms.
Activation of δ-cells via DREADD-Gq enhances glucose-dependent insulin secretion through rebound excitatory mechanisms that are partially mediated by somatostatin off-signaling. In contrast, δ-cell inhibition via DREADD-Gi modestly reduces somatostatin secretion but triggers a robust increase in glucagon release, with a comparatively limited effect on insulin output.

(A) Confocal image of a pancreatic islet (scale bar, 20 μm), and **(A1)** high-resolution image of a δ-cell (scale bar, 5 μm) showing HA-tagged DREADD-Gi expression (green) localized specifically to somatostatin+ cells (red).

(B–E) Dynamic perifusion profiles and AUC quantification of somatostatin secretion from islets expressing DREADD-Gi **(B, D)** or DREADD-Gq **(C, E)** following sequential 4-minute CNO pulses (1, 10, and 100 μM) under stimulatory glucose (16 mM). Each trace represents an individual islet preparation (n = 3 per group).

(F–H) Mean ± SEM hormone secretion profiles (n = 3 biological replicates per group) showing the effects of δ-cell activation (DREADD-Gq, green), inhibition (DREADD-Gi, red), or control (no DREADD, black) on somatostatin **(F)**, insulin **(G)**, and glucagon **(H)** secretion during perifusion in 1 mM and 16 mM glucose.

(I, J) Estimation of surplus insulin secretion in response to δ-cell activation versus inhibition. **(I)** Subtracted insulin secretion traces (mean ± SEM) from islets expressing DREADD-Gq (green) or DREADD-Gi (red), normalized to control traces. **(J)** AUC quantification of the traces shown in panel I.

(K, L) Mean ± SEM secretion profiles of insulin (black) and glucagon (gray) following δ-cell activation (**K**, green) or inhibition (**L**, red), highlighting reciprocal hormone responses.

(M, N) δ-cell activation–induced rebound insulin secretion is glucose-dependent. Perifusion traces **(M)** show insulin responses to two 2.5 μM CNO stimulations at 3 mM (light green), 7 mM (medium green), and 16 mM (dark green) glucose; **(N)** corresponding AUC quantification confirms dose dependence.

(O, P) Rebound insulin secretion induced by δ-cell activation at 16 mM glucose is partially inhibited by the somatostatin receptor antagonist cyclosomatostatin (200 nM). **(O)** Insulin secretion profiles (n = 8 biological replicates; 4 male, 4 female) from DREADD-Gq-expressing islets treated with CNO alone (green) or CNO + cyclosomatostatin (red-filled symbols), compared to untreated controls (black). **(P)** AUC analysis quantifying the effect of somatostatin receptor blockade on insulin rebound magnitude.

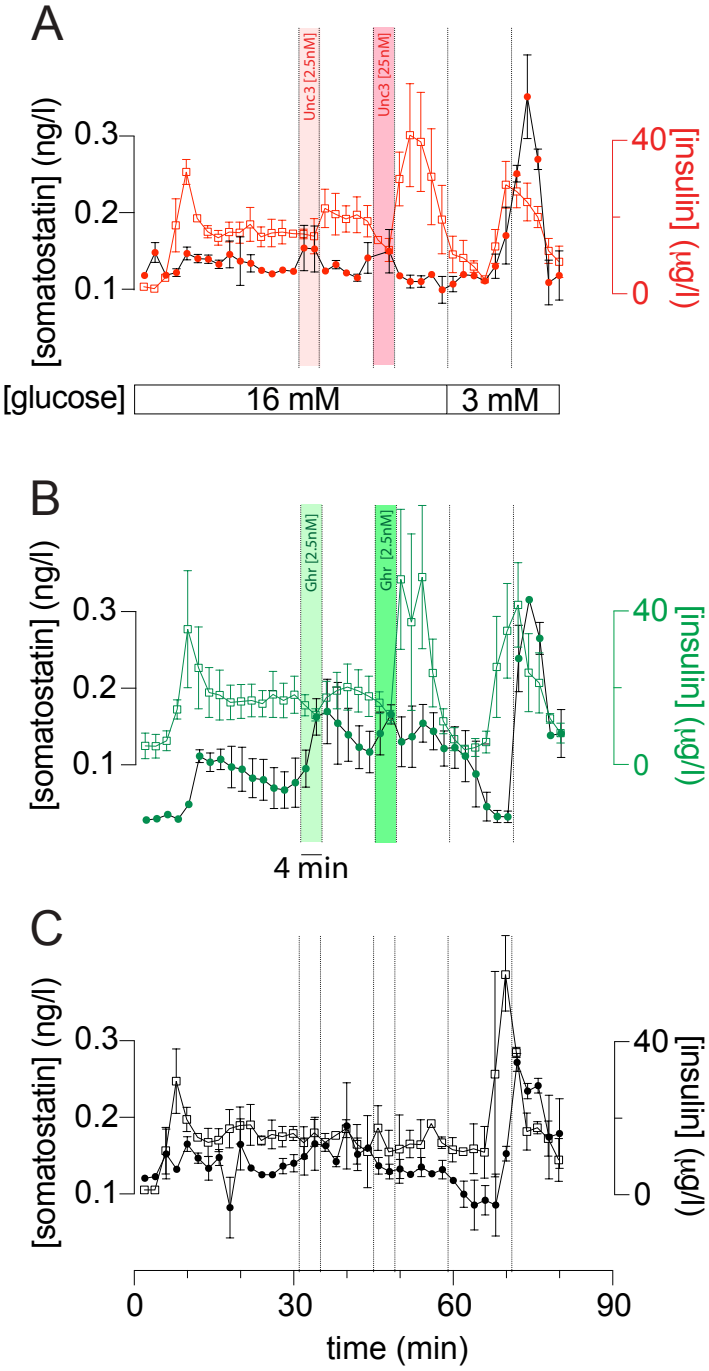

Figure 4. Endogenous δ-Cell-Targeting Ligands Unc3 and Ghr Elicit Dose-Dependent Somatostatin Secretion and Subsequent Insulin Rebound Responses.
Native ligands Unc3 and Ghr selectively activate δ-cells, triggering somatostatin release followed by secondary insulin rebound responses in a dose-dependent manner.

Perifusion assays were conducted on isolated pancreatic islets from 4-month-old male C57BL/6 mice (n = 6, Harlan Laboratories), cultured for 48 hours prior to experimentation. Islets (150 IEQ per column, 3 columns per condition) were perifused at 60 µL/min, with effluent collected every 2 minutes. All preparations were initially stimulated with 16 mM glucose to induce biphasic insulin secretion.

(A-C) Islets were then subjected to sequential 4-minute ligand pulses, spaced 10 minutes apart, using either 2.5 or 25 nM Unc3 (A), Ghr (B), or vehicle (sham control, C). Curves represent mean dynamic secretion profiles of somatostatin (filled symbols) and insulin (open symbols) across replicates.

Ligand-induced somatostatin release was followed by delayed, dose-dependent elevations in insulin levels, indicating a rebound secretory response. These data demonstrate functional engagement of δ-cells by Unc3 and Ghr and reveal a coordinated paracrine mechanism influencing β-cell output.

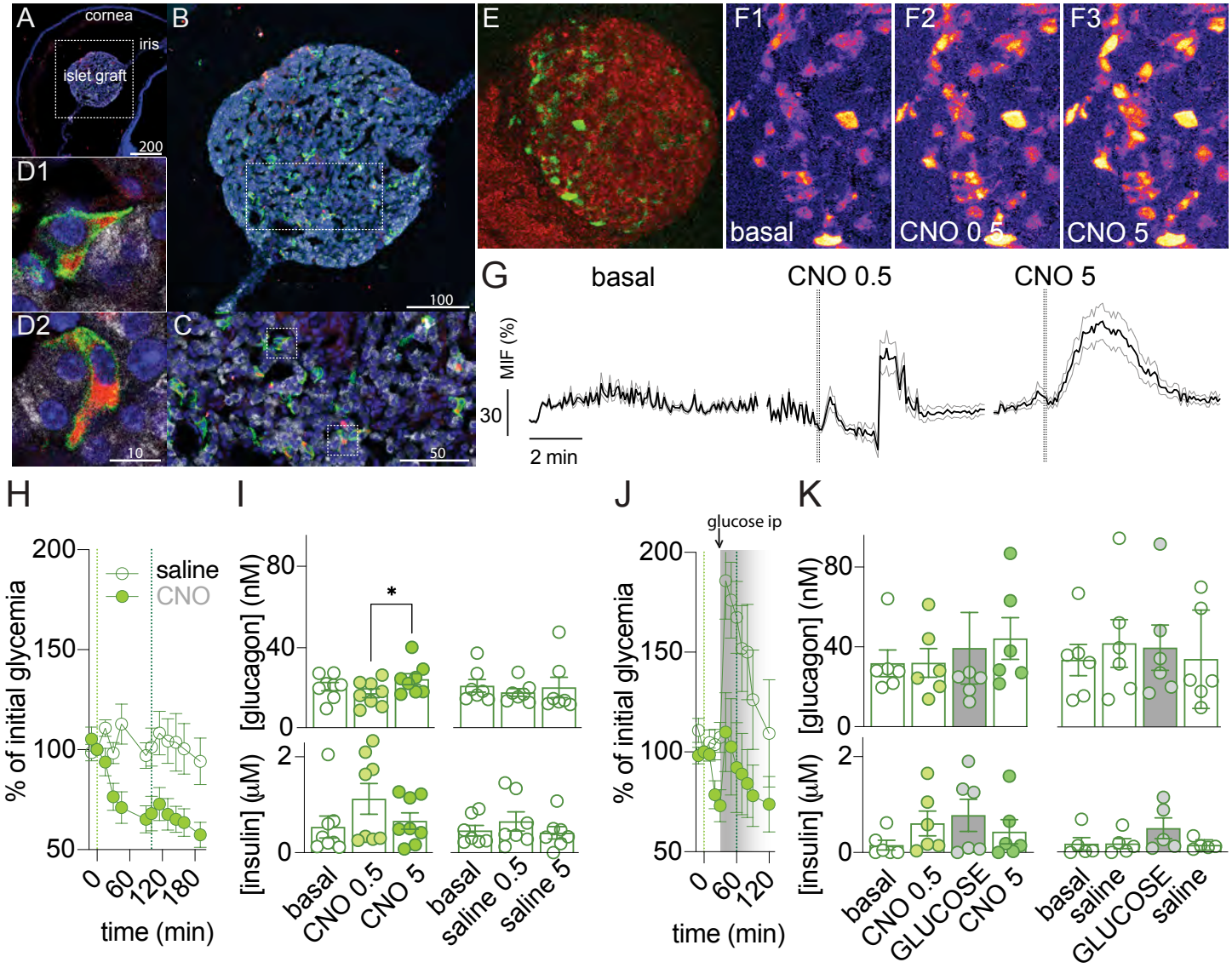

Figure 5. Repetitive in vivo activation of DREADD-Gq positive δ-cells in intraocular islet grafts via systemic CNO administration.
Proof-of-concept demonstration that DREADD-Gq positive δ-cells can be repeatedly stimulated in vivo through systemic administration of clozapine-N-oxide (CNO), resulting in dynamic intracellular Ca^{2+} responses and a concurrent hypoglycemic effect.

(A–D) Immunocytochemistry (ICC) of intraocular islet grafts transplanted onto the iris confirms persistent, δ-cell-specific expression of the DREADD-Gq reporter epitope HA (green) colocalized with somatostatin (SST, red). (A) Whole-eye image showing engrafted islet in the anterior chamber (scale bar, 200 μm). (B) Higher magnification of the grafted islet (scale bar, 100 μm). (C) Visualization of islet parenchyma (scale bar, 50 μm). (D1, D2) High-resolution images illustrating membrane-localized HA expression in individual SST^{+} δ-cells (scale bar, 10 μm).

(E, F1–F3) In vivo confocal Z-stacked 3D imaging of intraocular islet grafts expressing the cytosolic Ca^{2+} sensor GCaMP3 in δ-cells enables real-time monitoring of intracellular Ca^{2+} dynamics. Repeated intraperitoneal (ip) injections of CNO (0.5 and 5 mg/kg, indicated by dashed lines) result in distinct, time-locked increases in GCaMP3 fluorescence intensity, reflecting δ-cell activation (see Supplementary Video).

(G) Quantification of GCaMP3-derived fluorescence (ΔF/F) across time confirms the capacity for repetitive CNO δ-cell stimulation in vivo. Data represent mean ± SEM of normalized fluorescence (ΔF/F) from individual δ-cells across multiple islet grafts (n = cells, islets, and/or animals, as specified).

(H–K) CNO-mediated activation of DREADD-Gq^+ δ-cells induces robust and reproducible hypoglycemia in vivo. Mice receiving ip CNO injections (green symbols, G and I) exhibit significantly reduced blood glucose levels compared to saline-injected controls (open symbols). (H, J) Hypoglycemic responses were observed both under basal (non-fasting) conditions and (J) following ip glucose administration, with gray shaded area indicating glucose excursion post-glucose challenge. (I-K) Corresponding plasma insulin and glucagon levels measured following CNO administration reveal a trend toward elevated insulin levels, supporting the physiological relevance of δ-cell activation.

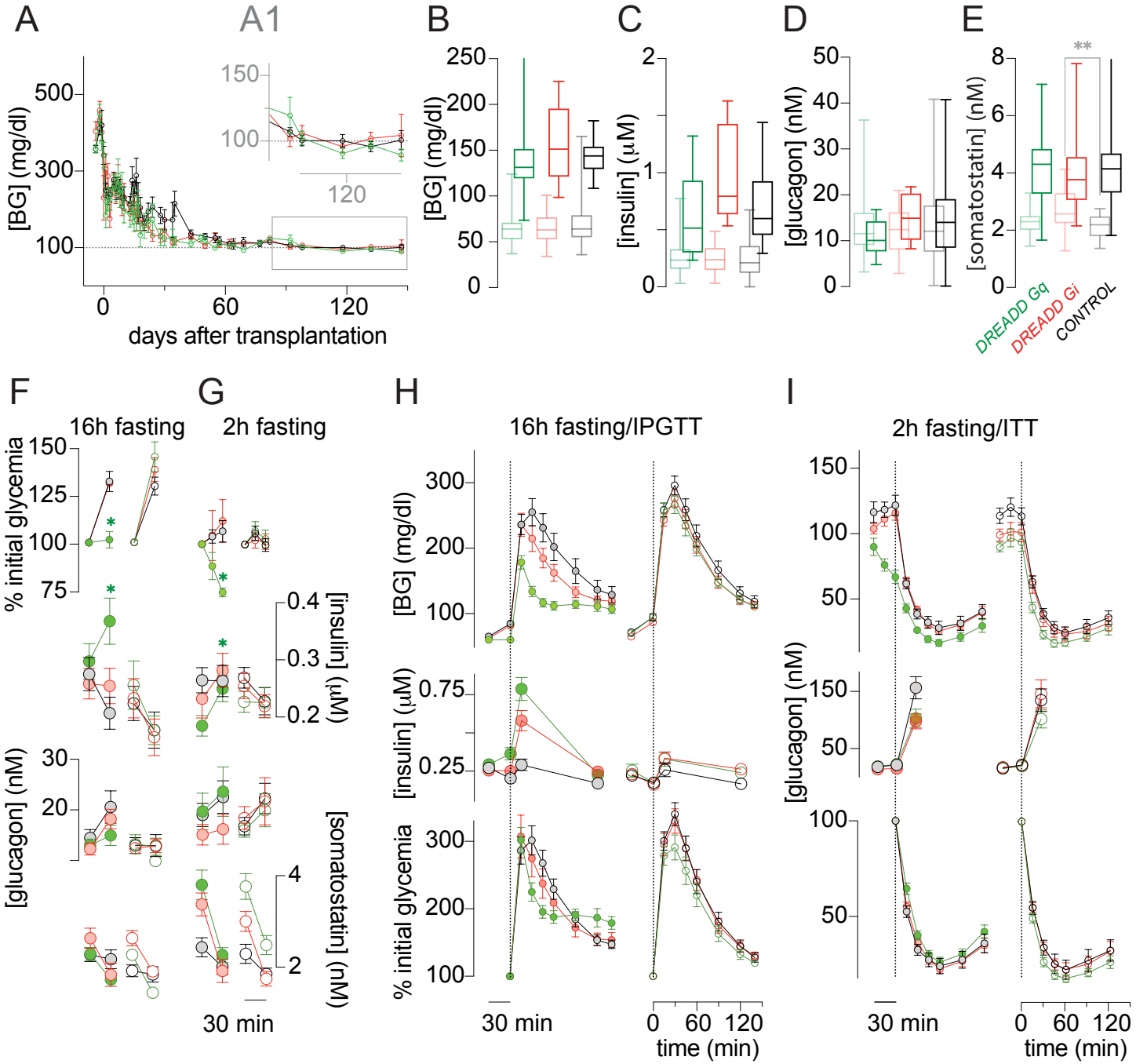

FIGURE 6. Acute activation, rather than inhibition, of islet δ-cells enhances insulin secretion and improves glucose handling in vivo

Metabolic profiling of mice with islets expressing either activator- (green), inhibitor- (red) DREADDs, or no-DREADD (black) in delta cells reveals that CNO-induced activation of δ-cells significantly increases plasma insulin levels. This leads to the expected hypoglycemic effect and improved glucose tolerance.

(A) - Follow-up of non-fasting glycemia (Mean ± SEM traces) in STZ-induced diabetic female NUDE mice shows that transplantation of isolated islets carrying DREADD Gq (green), DREADD Gi (red), or no-DREADD controls (black) restores normoglycemia and reverses experimental diabetes. These effects persist without significant difference after three months (zoomed-in A1, gray). Data are consolidated from two cohorts of recipient female mice transplanted with islets from either female or male donors per condition (n ≥ 7 per cohort).

(B-E) - Box-and-whisker plots comparing fasting (light border) and prandial/postprandial (dark border) conditions for glycemia (B) and plasma hormone levels of insulin (C), glucagon (D), and somatostatin (E).

(F-I)- Effects of 30-minute δ-cell stimulation via ip CNO injection (filled symbols) on glycemia and plasma hormone concentrations (insulin, glucagon, and somatostatin) were compared to control mice treated with saline (open symbols). CNO-induced activation of δ-cells significantly increased basal plasma insulin and induced a hypoglycemic response in both long-fasted (F) and short-fasted (G) mice, while no significant changes were observed in control or CNO-inhibited δ-cell mice.

(H) - Plasma insulin concentrations significantly increased following glucose injection during IPGTT, whether δ-cells were activated (green) or inhibited (red). However, insulin levels rose more markedly with δ-cell activation, and only activation of δ-cells improved glucose tolerance, as shown by both absolute blood glucose values and percentage change from baseline glucose levels after glucose injection.

(I)- Insulin tolerance tests were performed to assess how δ-cell manipulation affects insulin sensitivity, glucagon secretion, and glucose counterregulatory responses during insulin-induced hypoglycemia.

CNO-induced activation of δ-cells led to significant hypoglycemia, accompanied by elevated insulin levels, without notable changes in glucagon or somatostatin levels. Conversely, δ-cell inhibition had a more modest effect on glucose metabolism. These findings underscore the critical role of δ-cell activation in regulating insulin secretion and glucose homeostasis, highlighting its potential as a therapeutic target for improving glucose control in diabetes.

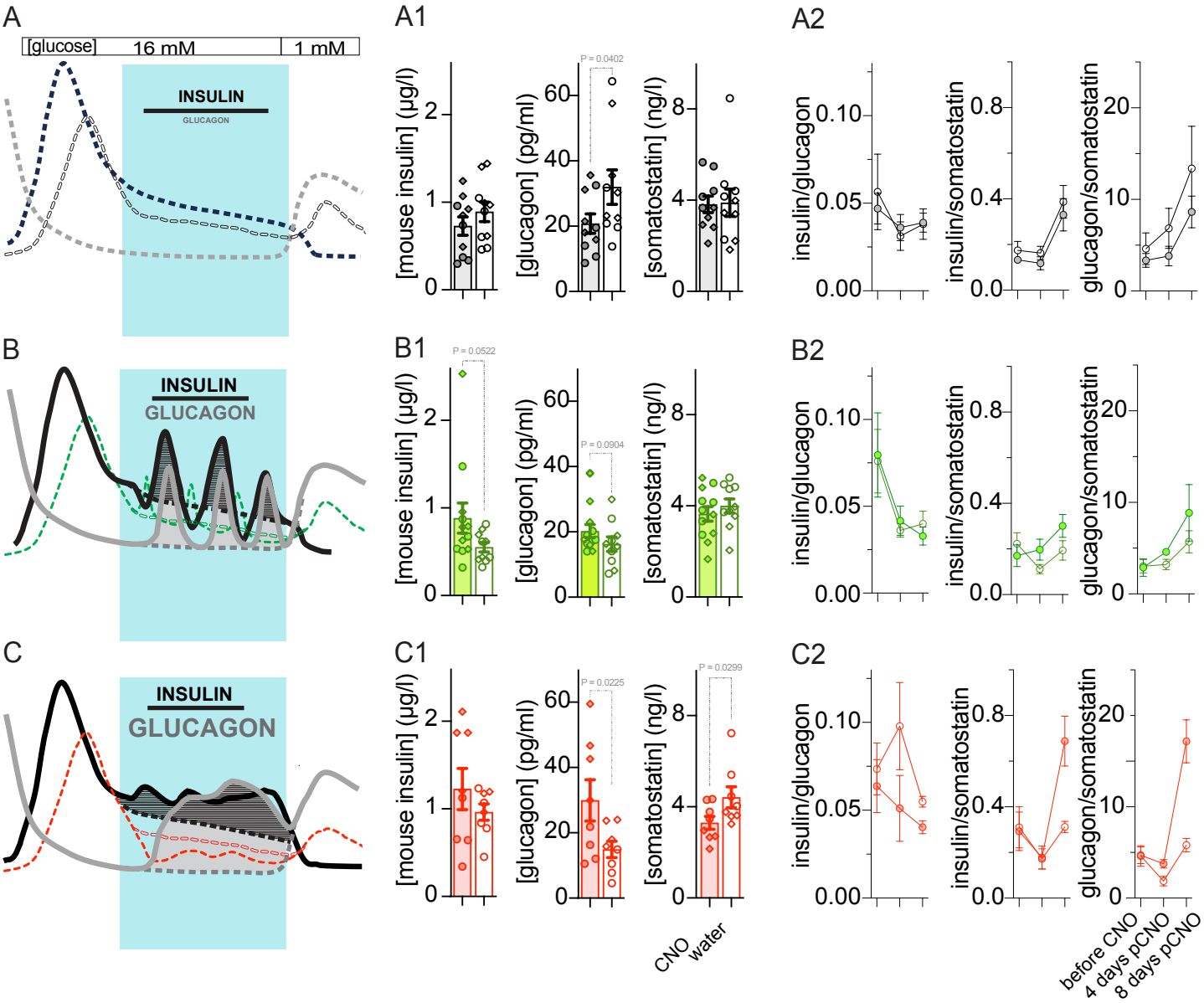

Figure 7. In vivo manipulation of islet δ-cell activity during feeding alters postprandial plasma levels and ratios of insulin, glucagon, and somatostatin in a manner consistent with in vitro perifusion studies.

Previous perifusion experiments showed that both activation and inhibition of δ-cell activity in the presence of 16 mM glucose enhanced insulin and glucagon secretion, but affected their balance in distinct ways. These in vitro findings suggested that δ-cell activity modulates the relative secretion of insulin and glucagon under high-glucose conditions.

To test these observations in vivo, we chronically manipulated δ-cell activity in feeding mice by administering CNO through drinking water, enabling selective activation or inhibition of DREADD-expressing δ-cells. Plasma hormone levels and their ratios further supported in vitro predictions: both activation and inhibition of δ-cells increased insulin and glucagon secretion, but activation favored a more balanced response skewed toward insulin, whereas inhibition disproportionately elevated glucagon.

(A–C) Schematic representations summarizing in vitro perifusion results (see also **Supplemental Figure S9**): (A) typical biphasic insulin response (dotted black) and glucose-mediated glucagon suppression (dotted gray) under high glucose; (B) hormonal changes during δ-cell activation (green dotted line); and (C) hormonal changes during δ-cell inhibition (red dotted line). Each stimulation condition was applied during high glucose (blue area), following the initial insulin response phase.

(A1–C1) Scatter plots showing individual data points and mean ± SEM plasma concentrations of insulin, glucagon, and somatostatin in prandial and postprandial states. NUDE mice transplanted with islets expressing either no DREADD (A1, black), excitatory DREADD (B1, green), or inhibitory DREADD (C1, red) were divided into subgroups receiving either water (open bars/symbols) or CNO in drinking water (filled bars/symbols). Hormone levels were measured after 3 (circles) and 8 (squares) days of treatment.

(A2–C2) Corresponding plasma hormone ratios (insulin:glucagon, insulin:somatostatin, and glucagon:somatostatin), presented as mean ± SEM, in the same animals before and after treatment. Filled symbols represent CNO-treated mice; open symbols indicate water-only controls.

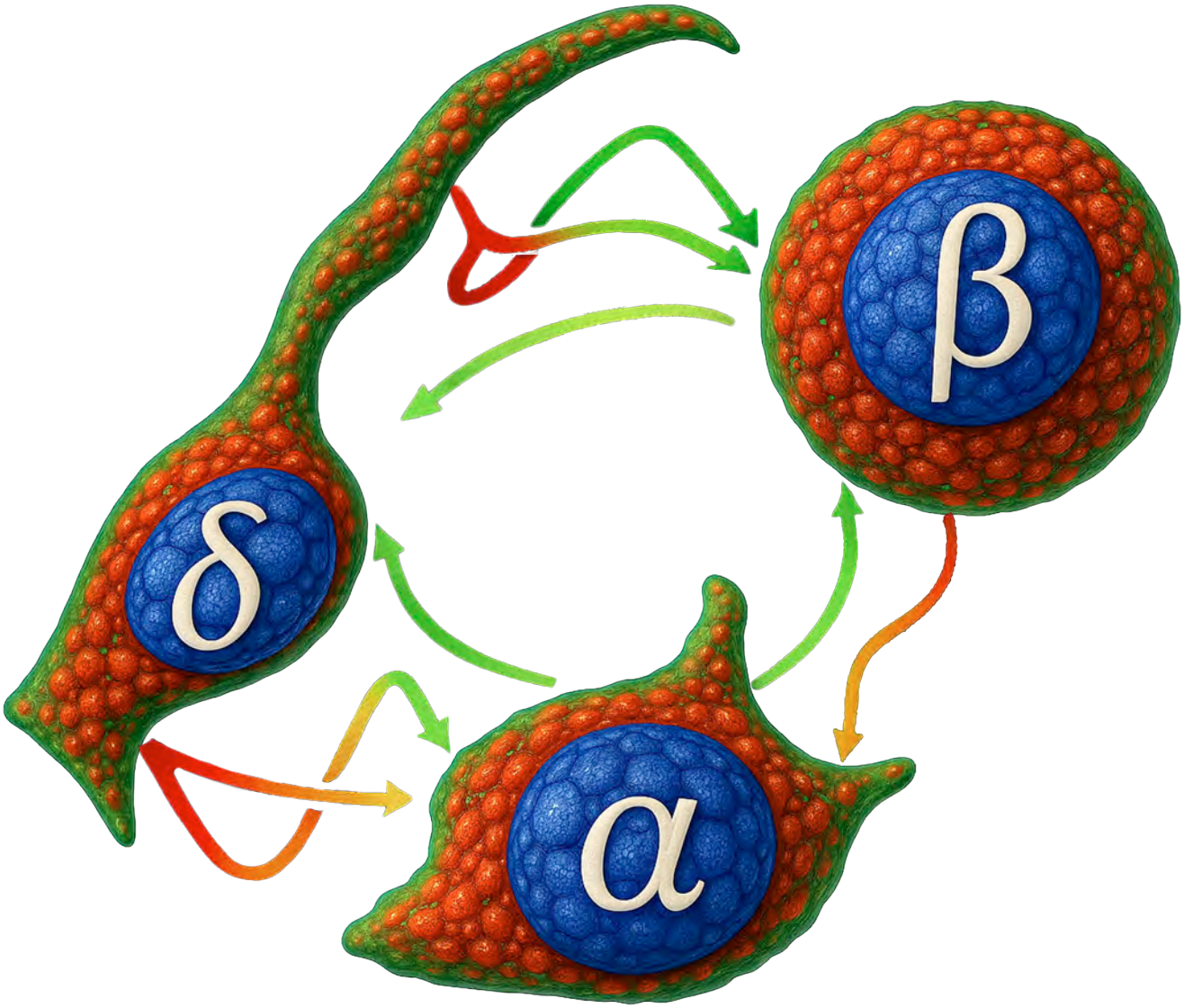

Figure 8. Emerging Model of Post-Inhibitory Rebound (PIR) Signaling and Dynamic Paracrine Crosstalk within the Pancreatic Islet

This schematic presents a revised model of intra-islet communication in which inhibitory interactions can transition into post-inhibitory rebound (PIR) excitation. δ-cells (green membrane, blue nucleus) exert temporally structured inhibitory control over β- and α-cells (red segments), followed by rebound excitation (green segments) that synchronizes insulin and glucagon secretion. β-cells, classically viewed as inhibitors of α-cells, also display evidence of PIR-type signaling (red-to-orange arrow), suggesting that rebound dynamics extend beyond δ-cell pathways. Straight green arrows denote established excitatory interactions independent of PIR. Collectively, these mechanisms redefine the pancreatic islet as a rhythmic, bidirectional network in which δ- and β-cell–derived signals transform inhibition into adaptive excitation, conferring endocrine plasticity and metabolic resilience.